



**PHARMACOGNOSTICAL & PRELIMINARY PHYTOCHEMICAL SCREENING,
MACERATION METHOD OF LAWSONIA INERMIS STEM**

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ABSTRACT

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Micromorphological characters for Lawsonia inermis stem are reported. The main constituent of Lawsonia inermis stem are Alkaloids, carbohydrates, glycosides, fixed oils & fats, flavonoids. It is characterized by the presence of trichomes. Epidermis is the outermost layer and consists of single layer epidermis of quadrangular cells. The outer walls of the cells are cutinised. From some of the cells multicellular, uniseriate hairs called epidermal hairs,(trichomes)are found. stomata are present. Cortex is well developed with collenchymatous, general cortex, hypodermis & endodermis as starch sheath. Pericycle is represented by semilunar patches of sclerenchyma with intervening masses of parenchyma. Vascular bundles are limited in number arranged in a ring & are wedge or top shaped and open. Xylem vessels are more in number & arranged in serial order. Medulla and medullary rays are present. From maceration method ethanol extract is more. Phytochemical investigation of stem is done with the ethanol extract. In ethanol extract good quantity of alkaloids, acid, coumarin, carbohydrate, glycosides, fixed oils fats, flavonoids, furonoids, phenols, phytosterols, triterpenoids, tannins, sterols, saponins are present.

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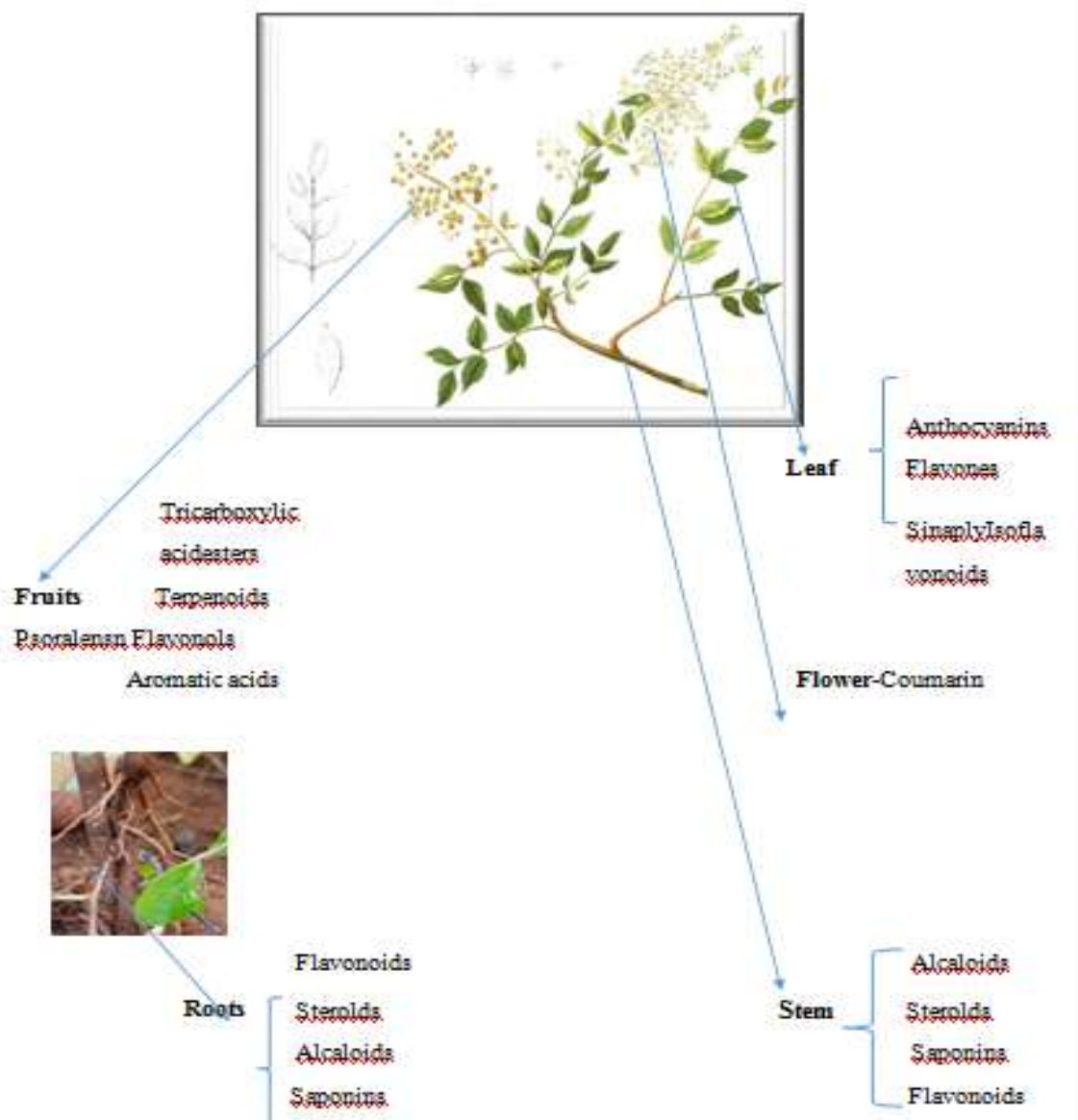
INTRODUCTION

Lawsonia inermis linn. Genus lawsonia bears one species, L. inermis (Henna, mehendi, shudi, maghati, madayantika & Gorinta) till date having different synonyms as alba & spinosa. It is a biennial dicotyledonous herbaceous shrub. A native of north Africa & south – west Asia, the plant is now widely cultivated throughout the tropics as an ornamental and dye plant. A much branched globular shrub or small tree (2 to 6 m in height).

TAXONOMY

- | | |
|---------------|---------------------|
| 1. Kingdom - | Plantae |
| 2. Family - | Lythraceae |
| 3. Order - | Myrtales |
| 4. Subfamily- | Lythroideae |
| 5. Genus - | Lawsonia |
| 6. Clade - | Rosids, Angiosperms |

**Delineate Structure of Lawsonia Inermis
plant**



ROOTS:

(Picbyyourself)

Henna a well known ethnomedicinal plant used cosmetically and medicinally in the Indian traditional folk medicines for thousand years. Root is used as Abortifacient, to treat leprosy , skin diseases dysmenorrhoea &premature graying of hair.

STEM:



(Picb yourself)

Evergreen shrub or tree 2–7 m high; young stems 4-sided, sometimes forming rigid spines Lawsonia inermis is a much-branched glabrous shrub or small tree 2-6 m in height, which may be spiny. Bark greyish-brown, unarmed when young, older plants with spine-tipped branchlets. Young branches quadrangular green but turn red with age.



LEAFS:

(Picbyyourself)

The leaves of henna, Lawsonia inermis, have been valuable since the olden days to beautify and dyeing the hands and feet to express tints of dark red, and for the controlling of definite skin disorders. The compound, lawsone, a brown powder isolated from the leaves impart their color in henna.

The paste of ground leaves has been used to colour skin, hair, fingernails, leather, and silk and wool.



FLOWERS :

(picbyGoogle search)

Henna flowers have four sepals and a 2 mm (0.079) in calyx tube , with 3mm (0.12) in



spread lobes .The ovary is four – celled,

5mm (0.20) in long and erect .Its petals are



ovate , with white or red stamens found in pairs on the rim of the calyx tube .The flowers and fruits are used in perfumery.

FRUITS:

(picbygooglesearch)

Henna fruits are small, brownish capsules, 4–8 mm (0.16–0.31 in) in diameter, with 32–49 seeds per fruit, and open irregularly into four splits. Fruits are also used for perfumery. Perfumery is the activity or business of producing perfume the perfumery trade. A perfumery is a store or



a department in a store where perfume is the main product sold.

Benefits & uses of Lawsonia Inermis (Henna)

- 7. Treats high fever
- 8. Dermatitis Remedy
- 9. Dye plant (red colour)
- 10. Relieves Headache
- 11. Sore Throat Treatment

The major constituent in Lawsonia inermis (Henna)

The traditional plant – derived henna contains the active ingredient called Lawsone (2-hydroxy-1,4 naphthoquinone). which is an orange – red pigment responsible for the typical coloration .

Lawsonia interacts directly with the amine or sulfur functional groups of Keratin due to its



Olden days in formation about lawsonia inermis

In Antient days(L.I) Henna leafs are used as{GORINTAKU}

Tillup to date also(L.I)henna leafs are still used as{GORINTAKU}

Gorintaaku is used on the hands from the ancient days because henna leaves are having skin carerotic an which helps to remove heat temperature from the body

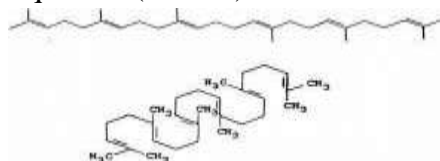


Chemical constituent of Lawsonia inermis—from traditional use

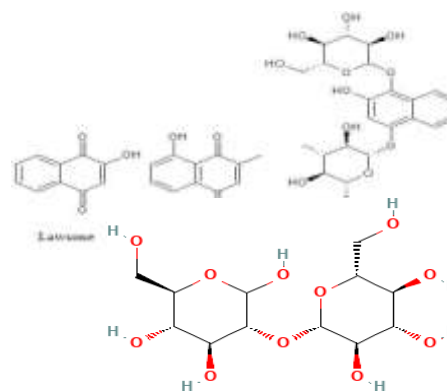
Beta-D-glucopyranoside

Octadecatrienoic acid (7.31%)

Squalene (7.20%)

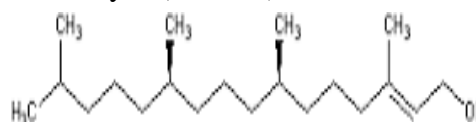


strong affinity.

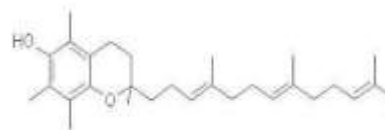


VitaminE (6.82%)

12.5.Phytol(10.85%)



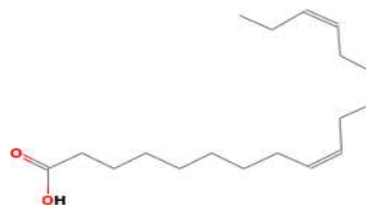
This are having major phytochemicals constituents in lawsonia inermis.



MATERIALS &METHODS

Collection & Identification:

The proposed material for study was identified and submitted as Lawsonia inermis L stem and it as authenticated by Dr. K. Madhava chetty. Assistant professor, Department of Botony, Srivenkateshwara university, Tirupati and voucher no. 0397.Lawsonia inermis stem



were collected from around Tirupati – 517 502 on 28/09/2023. The stem were collected washed with water, dried in sunlight and stored properly. The dried stem was powdered. Coarse powder was used for phytochemical and maceration work.

Macroscopy studies:

The term macroscopy refers to things that can be seen with the naked eye. The macroscopical characters like size, shape, base, surface, color, odor and taste of Lawsonia inermis.

Microscopy studies:

The term microscopy refers to object that are so small that they can be observed only with the help of microscope. The required samples of Lawsonia inermis L. stem were sectioned with the help of fresh blade. The sections were first cleared with chloral hydrate and then stained with phloroglucinol and concentrated HCL sections were also stained with Iodine solution (I-KI) for starch. Photographs were collected or taken with Binocular microscope observation.

Maceration studies:

Maceration is one of the simplest extraction technique in which coarse and powdered plant material is soaked such as methanol, ethanol, ethylacetate, acetone, hexane etc. It is one of the popular and in expensive technique used for the extraction of different bioactive compounds from plant material.

Solvents used :

13. Water
14. Ethanol
15. Methanol
16. Chloroform

Procedure: Fresh stem of Lawsonia inermis were collected & dried for 3-5 days under the sunlight. Dried stem are grinded to convert into powder. Weigh the 10 gm of dried powder of (LI) stem and add 10 gm of powder into each 200 ml of water, ethanol, methanol, chloroform solvents mix each solvent with L stem powder & cover them with a Aluminium foil paper. Keep it aside for 48 hours and filtrate it.

Preliminary phytochemical screening:

The EELI (Ethanol extract of Lawsonia inermis) obtained was subjected to different qualitative chemical tests for the identification of different chemical constituents.

Detection of Alkaloids:

Dragendroff's reagent:

A few mgs of EELI inactive acid or HCL was treated with two drops of Dragendroff's reagent (potassium mercuric iodide).

Wagner's reagent:

A few mgs of EELI was treated with two drops of Hager's reagent.

Acids: A few mgs of EELI was treated with aqueous NaHCO₃.

Detection of Coumarin:

A few mgs of the EELI in alcohol was treated with alcoholic NaOH.

Detection of Carbohydrates :

A few mgs of the EELI was dissolved in suitable solvent and filtered. The filtrate was subjected to the following tests.

Molish Test:

The filtrate was treated with 2-3 drop of 1% alcoholic naphthol and 3 ml of conc. H₂SO₄ was added along with the sides of the test tube.

Fehling Test:

The filtrate was first treated with 1 ml of Fehling's test solution and heated. The reddish or orange precipitate was obtained. The red precipitate indicates the absence of reducing sugars. Another portion of liquid hydrolysate was subjected to "Legals" and Borntragers' test.

Legals Test:

To the hydrolysate 1 ml of sodium nitroprusside solution was added and it was made alkaline with NaOH solution.

Borntragers Test:

The hydrolysate was treated with chloroform layer was separated. Equal quantity of dil. Ammonia solution was added to it.

Detection of Fixed oils & fats:

A few mgs of EELI was pressed separately between two filter papers. If characteristic oil stain was observed.

Phenolphthalein Test:

A few drops of alcoholic KOH added to the EELI with a few drops of phenolphthalein. The mixture was heated on a water bath for one to two hours. The formation of soap was observed.

Flavonoids:

Shinoda Test:



A few mgs of EELI was dissolved in alcohol separately and was treated with magnesium foils and a few drops of concentrated Hydrochloric acid.

17. A few mgs of EELI was dissolved in separate alcohol and to this a Magnesium metal, followed by concentrated Hydrochloric acid added to the solution.

18. A small portion of the EELI was dissolved separately in alcohol. The EELI was yellow in colour which on addition of acid becomes colourless.

Detection of Furonoids:

Ehelich Test:

A few mgs of EELI was dissolved separately in alcohol and was treated with a pinch of para dimethylamino-benzaldehyde and a few drops of hydrochloric acid.

Detection of Phenols:

A few mgs of EELI was dissolved separately in alcohol and was treated with alcoholic ferric chloride.



Detection of Phytosterols:

A few mgs of EELI was dissolved in 5 ml of chloroform separately. Then this chloroform solution was subjected to a Liebermann-Buchard test. The above prepared chloroform solution was treated with a few drops of concentrated Sulphuric acid followed by 1 ml of active anhydride solution. This solution was heated gently if necessary.

Salkowski's Test:

To 1 ml of above prepared Chloroform solution and a few drops of concentrated H_2SO_4 was added.

Detection of Saponins:

A few mgs of EELI was diluted with 20 ml of distilled water and it was heated in a graduated cylinder for 15 minutes.

Detection of Tannins:

A few mgs of EELI was dissolved in alcohol separately and it was treated with a few drops of aqueous lead solution.

Detection of Triterpenoids:

A few mgs of EELI was taken in a dry test tube and it was treated with a bit of tin foil and 0.5 ml of thionyl chloride. Heated gently if necessary.

RESULTS

Macroscopical characters:

The stem of Lawsonia inermis L is a much-branched glabrous shrub or small tree 2-6 m in height, which may be spiny. It is a ruff-like structure. Bark greyish-brown, unarmed when young, older plants with spine-tipped brachlets. Young branches quadrangular, green but turn red with age. Fig 1 shows photograph of Lawsonia inermis stem.

Fig1 Photograph of lawsonia inermis stem Microscopical characters:

A transverse section of stem from Lawsonia inermis plant is circular in outline and differentiated into three regions they are epidermis, cortex, and stele.

Epidermis:

It is the outermost layer and consists of a single layer of epidermis of quadrangular cells. The outer walls of the cells are cutinised. From some of the cells multicellular, uniseriate hairs called epidermal hairs, (Trichomes) are formed. Stomata are present.

Epidermis Layer

It is the middle region and consists of three parts they are Hypodermis, middle cortex and stele.

Hypodermis:

Below the epidermis 6-7 layered collenchymatous hypodermis is present. It gives mechanical support.

Innercortex:

It consists of 7-10 layer of loosely arranged parenchymatous cell filled with chloroplasts. In this region small

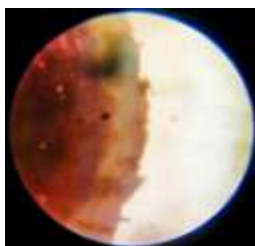


mucilage ducts are present.

Endodermis:

Below the innercortex single layered endodermis are present. Endodermis cells contain starch grains. so called starch sheath.

Stele:



Stele is the inner region and occupies relatively large region in stem. It consists of pericycle, vascular bundle sheath & medullary rays. Pericycle is present as 3 to 6 layered sclerenchymatous patch above each vascular bundle with intervening masses of parenchyma.

Vascular bundles are 15-20 in number and are arranged in a single ring (Eustele). They are top shaped, conjoint, collateral and open.

Medullary rays:

Parenchymatous tissue present between vascular bundles helps in lateral conduction. Medullary rays arising from pith region.

Pith:

Central part of the stem is occupied by parenchymatous pith. After pith region xylem and phloem are present.

Identification characters: Nodes and

internodes are present. Cuticle and stomata are present. Hypodermis is collenchymatous. Cortex is smaller than stele. Connective tissue is present. Vascular bundles are conjoint & collateral. Xylem is



end arch



Maceration:

19. After filtering all solvents ethanol solvent got more solvent extract compare to the solvent extracts.

Phytochemical Analysis of Lawsonia inermis stem:

Detection of Alkaloids

Dragendorff's reagent: Red or orange colour precipitation indicated the presence of alkaloids.

Wagner's reagent: Reddish brown colour precipitation indicated the presence of alkaloids.

Hager's reagent: Yellow colour indicated the presence of alkaloids.

Acids

Effervescence shows the presence of acid, which was due to liberation of carbon dioxide.

Detection of Coumarin

Yellow colour indicated the presence of coumarin.

Detection of Carbohydrates

Molisch Test: The characteristic colour indicates the presence of carbohydrates.

Fehling Test: Legal's & Brontragers test to predict the presence of different glycosides.

Legal's Test: The change in characteristic colour indicates the presence of glycosides.

Brontragers Test: The change in the characteristic colour indicates presence of glycosides.

Detection of Fixed oils & Fats:

If a characteristic oil stain was observed indicates the presence of fixed oils & fats.

Phenolphthalein Test:

The formation of soap was observed indicates the presence of fixed oils & fats.

Flavonoids

Shinoda Test:

Red or pink colour indicates the presence of flavonoids.

20. This slowly turned to intense yellow colour and then dark pink colour.

21. Thus it confirms presence of flavonoids.

Detection of Furonoids

Helich Test: It forms red or pink colour and this indicates the presence of furonoids.

Detection of Phenols:

Any characteristic colouration indicates the presence of phenols.

Detection of Phytosterols:

The formation blue or green colour shows the presence of sterols.

Salkowskis Test :

The formation of brown colour indicates the presence of phytosterols.

Detection of Saponins

The formation of foam shows the presence of saponins.

Detection of Tannins

The formed precipitation indicates the presence of tannins.

Detection of Triterpenoids

The formation of pink colour shows the presence of triterpenoids.

DISCUSSION

Lawsonia inermis L. stem main chemical constituent of Lawsonia inermis stem are Alkaloids, carbohydrates, glycosides, fixed oils & fats, flavonoids. It is characterized by the presence of trichomes. Epidermis is the outermost layer and consists of single layer epidermis of quadrangular cells. The outer walls of the cells are cutinised. From some of the cells multicellular, uniseriate hairs called epidermal hairs, (trichomes) are found. Stomata are present. Cortex is well developed with collenchymatous, general cortex, hypodermis & endodermis as starch sheath. Pericycle is represented by

semilunar patches of sclerenchyma with intervening masses of parenchyma. Vascular bundles are limited in number arranged in a ring & are wedge or top shaped and open. Xylem vessels are more in number & arranged in serial order. Medulla and medullary rays are present. In phytochemical investigation of stem in ethanol extract alkaloids, acids, coumarin, carbohydrates, glycosides, fixed oils & fats, flavonoids, furonoids, phenols, sterols, phytosterols, saponins, tannins, triterpenoids were present. Sugar were absent.

CONCLUSION

In these present investigations various pharmacognostical standardization parameters such as macroscopy, microscopy, maceration & preliminary phytochemical screening were carried out which could be helpful in authentication of Lawsonia inermis L. The result of the present study will also serve as reference material in the preparation of herbal monograph.

REFERENCES:

1. Swaroopa Rani N, Gupta: Review on Lawsonia inermis and its Applications Vol-5, 5-2018.
2. Sains Malaysiana : Optimization of extraction parameters of total phenolic compounds from Henna (Lawsonia inermis) Leaves. 39 (1) (2010): 119-128.
3. V.C. Jain, D.P. Shah, N.G. Sonani: Pharmacognostical & preliminary phytochemical investigation of Lawsonia inermis L. Leaf. V-55, No. 2, P. 127-133, Bucharest, 2010
4. Wasim Raja, M. Ovais & Amit Dubey: Phytochemical screening & Antibacterial activity of Lawsonia inermis leaf extract. Vol 4(1): 33-36, 2013.
5. L.C. Chuku, N.C. Chinaka : Phytochemical screening and Anti-inflammatory properties of Henna Leaves (Lawsonia inermis). Vol 31(18): 23-28, 2020.
6. Amina Moutawalli, Fatima Zahra

- Benkhouil: The biological and pharmacologic actions of Lawsonia inermis L. Vol 3, Issue 3, August 2023, 100468.
7. Amit S. Borade, Babasaheb N. Kale: A phytopharmacological review on Lawsonia inermis (Linn) Vol 2(1): Jan 2011, 536-541.
 8. Kruti M. Patel, Pratik R. Patel, Review on Lawsonia inermis Linn : An Update Vol 7(4): 2017, 237-250.
 9. Kamal M, Pharmacological Activities of Lawsonia inermis Linn: A Review Vol 1(2), 62-68: 2010.
 10. Haq Nawaz: Effect of solvent polarity on extraction yield and antioxidant properties of phytochemicals from bean (Phaseolus vulgaris) seeds. pp 02-02-2018.
 11. Kandiah Uthayarasa: Antibacterial activity and qualitative phytochemical analysis of Medicinal Plant extracts obtained by sequential extraction method. Pp. 14-06-2010.
 12. A.G. Patil, S.P. Koli & A.V. Phatak: Evaluation of extraction techniques with various solvents to determine extraction efficiency of selected medicinal plants. Vol 3(8): 2607-2612, 14 May 2012.
 13. Buddhadev S.G & Buddhadev S.S, Ayurvedic medicinal plant Lawsonia inermis Linn: A complete review, Vol 7(2), Apr-Jun 2016, 240-248.
 14. Amina Moutawalli, Fatima Zahra Benkhouili, Anass Doukkali, Hanane Benzeid: The biological and Pharmacological actions of Lawsonia inermis L. Vol 3(3), August 2023, 100468.
 15. Saloman Poliwoda, Nazir Noor, Evan Pown, Amanda Schaaf, Stem cells : A comprehensive review of origins and emerging clinical roles in medical practice. Vol 14(3), 2022: 37498.
 16. Badoni Semwal R, Semwal D K, Combrink C K.S, Cartwright-Jones C, Viljoen A: Lawsonia inermis L (henna): ethnobotanical phytochemical and pharmacological aspects, 02 Jun 2014, 155(1): 80-103.
 17. Dhuransure Rajkumar, Biradar, Sanjiv Kumar, Nanjwada BK, Patil MH, Sirse KK: Microscopical investigation of Roots of Lawsonia inermis Linn. Vol 5(3), 2013: 169-172.
 18. Snehal D. Kothowale, Asha K. Patil, Roshani P. Kumbhar, S.K. Mhite: A Review on Henna Vol 13(1) 2023.
 19. Ruchi Bodoni Semwal, Deepak Kumar Semwal, Sandra Combrink : Lawsonia inermis L (Henna) : Ethnobotanical, phytochemical and pharmacological aspects. Vol 155(1), 8 August 2014, 80-103.
 20. Emin Zumrutdal & Mehmet Ozaslan : A Miracle plant for the Herbal pharmacy; Henna (Lawsonia inermis). Vol 8(6), 2012: 483-489.